Effect of Ionizing Radiation on Unesterified Tocopherols in Fresh Chicken Breast Muscle

ABSTRACT

The effect of ionizing radiation on free tocopherols in chicken was determined. Raw chicken breast muscle with skin and adipose tissue removed was subjected to gamma radiation from a ^{137}Cs source at 1, 2·25, 5·0, and 10·0 kGy. The chicken was packaged aerobically, and irradiated at 4°C. Free tocopherols were extracted directly from the meat without a saponification step. The tocopherols were resolved using normal phase, high performance liquid chromatography by spectrophotofluorometric detection. Irradiation resulted in a significant linear decrease in alpha and gamma tocopherol with increasing dose levels. At 3 kGy, the maximum level approved by the FDA for poultry, a 15% reduction of free gamma tocopherol and a 30% reduction for free alpha tocopherol were observed.

INTRODUCTION

The United States Food and Drug Administration issued a ruling in 1990 permitting the commercial irradiation of fresh or frozen poultry, parts, or finely comminuted poultry up to a level of 3 kGy (Federal Register, 1990). The stated purpose for irradiation of poultry was to reduce food-borne pathogens. In anticipation of such a ruling, a study was initiated to determine the effects of ionizing radiation on the water- and fat-soluble vitamins. Fox *et al.* (1989) and Jenkins *et al.* (1989) have studied the effect of gamma (γ) radiation at this dose-level on water-soluble B vitamins. This study measures the effect on vitamin E, the most labile (Knapp & Tappel,

1961*a*) of the fat-soluble vitamins, and therefore the most sensitive indicator of the effects of radiation on this class of vitamins.

Vitamin E is a collective term used to describe the tocopherols ($\alpha, \beta, \gamma, \delta$) and their corresponding four tocotrienols. In humans, D-α-tocopherol is physiologically the most active tocopherol. Vitamin E is only formed *in vivo* by plants. It is present in animal tissue only as a result of the ingestion of plant material. Most of the earlier studies were conducted on products with high tocopherol levels or on model systems. Many of the studies conducted on model systems determined that the relative stability of α-tocopherol to ionizing radiation was a function of the solvent (Knapp & Tappel, 1961*b*; Rose *et al.*, 1961; Chipault & Mizuno, 1966; Gray, 1978; Diehl, 1979*a*). Investigations relating to the effect of radiation on vitamin E in foods were conducted mainly on grains and vegetable oils since these contain relatively high levels of tocopherol. Comprehensive studies performed by Diehl (1979*a*) measured such parameters as the effect of temperature, atmosphere, subsequent processing, duration of storage, and synergistic effects on the stability of irradiated tocopherol. Relatively few studies have been reported on the effect of ionizing radiation on the low levels of vitamin E present in meat (de Groot *et al.*, 1972; Diehl, 1979*b*; Roussel, 1988). In view of the recent approval to permit commercial irradiation of poultry, this study examines the effect of radiation on the vitamin E in chicken breast muscle under conditions similar to those recently approved. This research is part of a continuing investigation to measure the overall effects of γ radiation on the nutritional content and microbial populations in meat and poultry.

MATERIALS AND METHODS

Reagents

D-α-tocopherol (5,7,8-trimethyltocol), and D-γ-tocopherol (7,8-dimethyltocol) were used as purchased from Eastman Kodak Co. (Rochester, NY). D-δ-tocopherol (8-methyltocol) 90%, obtained from Sigma Chemical Co. (St Louis, MO), was purified using semi-preparative μ Porasil high performance liquid chromatography (HPLC) (Waters, Milford, MA). Butylated hydroxytoluene (BHT) (99%) was purchased from Aldrich Chemical Co. (Milwaukee, WI) and was recrystallized from methanol. BHT, an antioxidant, was added to cyclohexane at a concentration of 100 mg/l prior to extraction. All solvents used were 'distilled in glass grade'.

Materials

Whole chickens (fryers) were purchased 24–48 h after slaughter. The breast muscle was separated from the skin, all visible adipose tissue, and cartilage.

To obtain a homogeneous sampling, the muscle was diced and mixed. Grinding of chicken was avoided so as to maintain the integrity of tissue prior to irradiation and minimize air oxidative destruction of the tocopherols. The samples were prepared at 4°C and irradiated and extracted on the day of purchase, to reduce possible losses of vitamin E due to prolonged storage. The entire analysis was carried out without fluorescent lighting and in the absence of direct daylight.

Irradiation

All samples were sealed in oxygen-permeable (2500 ml/100 in^2/24 h) meat and poultry bags (Mobil Chemical Co., Macedon, NY). These were irradiated with a ^{137}Cs source (dose rate of 0·118 kGy/min) to levels of 1·0, 2·25, 5·0 and 10·0 kGy at temperatures between 4 and 6°C. Opti-chromic film dosimeters (Far West Technology Inc., Goleta, CA) were used to monitor the absorbed dose.

Extraction

Muscle tissue (20 g) was weighed into screw-capped centrifuge bottles (130 ml) containing 100 ml cyclohexane. An internal standard, 8 μg δ-tocopherol, was added. The lipids were extracted by homogenizing the tissue in a Polytron (Kinematica, Lucerne, Switzerland). The samples were centrifuged at 1500 × g for 5 min at 4°C with the resulting supernatant being retained and the centrifugate re-extracted with 75 ml cyclohexane. The combined supernatants were dried over anhydrous sodium sulfate, filtered through glass wool and diluted to 200 ml. An aliquot was transferred and dried under vacuum on a rotary evaporator at ambient temperature. The sample was reconstituted with iso-octane, centrifuged, and filtered (0·45 μm ARCO LC 13 (Gelman Science, Ann Arbor, MI)) into an autosampling vial and sealed.

High performance liquid chromatography

The HPLC instrumentation used to isolate and quantitate the tocopherols consisted of an Autochrom M500 pump (Milford, MA), a model AS-100 refrigerated autosampler (Bio Rad, Richmond, CA), and a Perkin–Elmer spectrophotofluorometer (Norwalk, CT) equipped with a xenon lamp and a 20 μl flow cell. Fluorometer settings were: E_x 292 nm, E_m 324 nm, cut-off filter 310 nm, and slits 5 nm (emission and excitation). Data were acquired using a SP 4290 integrator (Spectro Physics, San Jose, CA).

Separation of the tocopherol isomers was achieved using a 100 mm ×

3·0 mm i.d. Chromspher 5 μm Si column (Chrompack Inc., Raritan, NJ). A guard column (10 mm × 2·2 mm) packed with similar material (Si, 35 μm), was also used. The mobile phase was reagent grade iso-octane-tetrahydrofuran (THF) (98:2, v/v) degassed by filtering through a 0·45 μm filter (Supelco Inc, Bellefonte, PA) under vacuum. A constant helium sparge was maintained. The flow rate was 0·8 ml/min. Column and solvents were at ambient temperature. Under these conditions, analysis of the tocopherols was completed within 7 min (Fig. 1).

RESULTS AND DISCUSSION

Tocopherols are readily susceptible to air oxidation, a reaction that is accelerated by light, heat, alkali, and certain trace metal ions (Nelis *et al.*, 1985). Studies conducted by Diehl (1979*a*) and others have indicated that storage of irradiated products, especially at low temperatures, could result in loss of vitamin E due to the accumulation of hydroperoxides formed as a result of irradiation. Therefore, a simple, rapid, and direct extraction procedure was employed to minimize oxidative loss of vitamin E. In the current study the tocopherols were extracted by homogenizing the tissue with cyclohexane containing 0·01% BHT. Saponification was not employed in order to avoid possible destruction of the unesterified tocopherols.

Fluorescence detection was chosen to measure the concentration of the naturally fluorescing tocopherols because of the enhanced selectivity and sensitivity of fluorescence compared with absorbence detection. Few natural compounds have fluorescent characteristics similar to tocopherols (Duggan *et al.*, 1957). The selectivity of this detection system allowed for the direct injection of the cyclohexane extract following a concentration step without extensive clean-up. The fluorescence detectors could readily detect 5 ng of a tocopherol.

Delta tocopherol was chosen for use as an internal standard because it is not present in meat and preliminary studies confirmed that it is not formed as a radiolytic product. The internal standard was added prior to the initial extraction. This permitted normalization of the results to a common basis and compensated for losses occurring during sample handling and extraction. Use of an internal standard also allowed for adjustment for minor deviations in sampling by the auto analyzer, and for variations in solvent composition or flow rate.

Reverse phase chromatography, using a C 18 column, was initially used to isolate the tocopherols. However, the concentrated extract had limited solubility in the mobile phase (methanol). Normal phase chromatography on a 10 cm Si column with an iso-octane/THF (98/2) mobile phase resulted

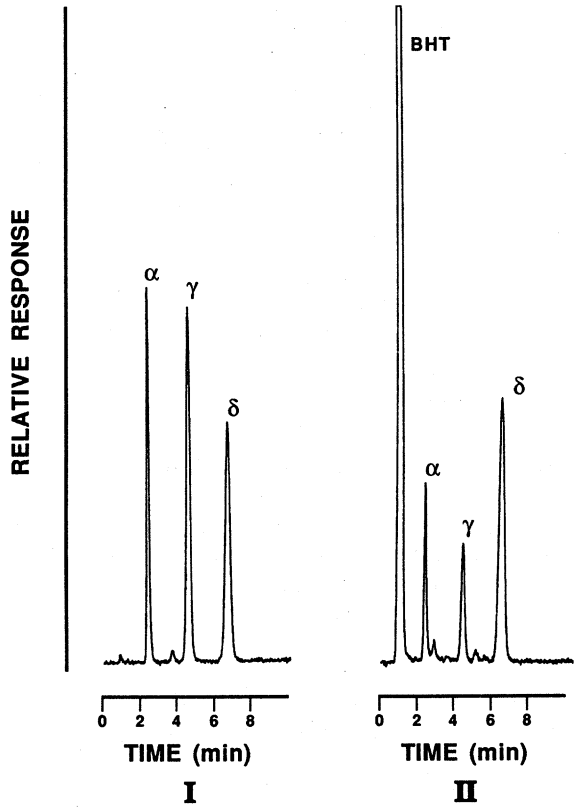

Fig. 1. HPLC chromatograms of: (I) tocopherol standards and (II) chicken breast extract irradiated at 10·0 kGy dose level. Chromatographic conditions: Column: Chromspher Si 5 μm, 100 × 3·0 mm i.d.; Eluent: iso-octane/tetrahydrofuran (98:2, v/v); Flow rate: 0·8 ml/min; Detection: fluorescence; E_x 292 nm, E_m 324 nm; Peak identification: butylated hydroxytoluene (BHT), α-tocopherol, γ-tocopherol, and δ-tocopherol. Internal standard (δ-tocopherol) and BHT are both additives.

in rapid analysis and made possible the differentiation of positional isomers. Chromatography of the chicken extract, control and irradiated (Fig. 1), indicated the presence of α- and γ-tocopherol in breast muscle as determined by retention times and confirmed by co-injection of tocopherol standards.

Analysis of the samples by HPLC included periodic injection of a standard solution (20 ng/μl of α-, γ-, and δ-tocopherol dissolved in iso-octane with 0·01% BHT) in order to determine detector response, constancy of response, retention times and quality of separation. The concentrations of tocopherols in the meat samples were calculated by comparing their peak areas to those of the standard calibration curve. A linear relationship between peak area and concentration of tocopherols existed within the concentration range studied (0–300 ng) and is shown in Fig. 2. Correlation

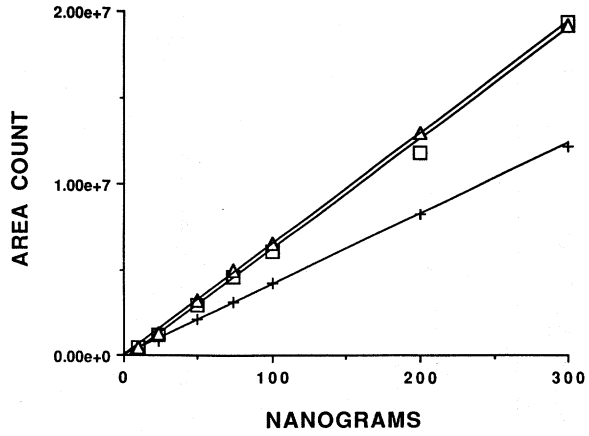

Fig. 2. Relative response of tocopheral standards chromatographed on Chromspher Si with fluorescence detection. Concentration of standard mixture 2 ng/μl and 20 ng/μl. Concentration range tested: 0–300 ng. Chromatographic conditions: Column: Chromspher Si 5 μm, 100 × 3·0 mm i.d.; Eluent: iso-octane/tetrahydrofuran (98:2, v/v); Flow rate: 0·8 ml/min; Detection: fluorescence; E_x 292 nm, E_m 324 nm; Peak identification: +, α-tocopherol; △, γ-tocopherol; □, δ-tocopherol.

coefficients of 0·9987, 0·9991, and 0·9696 for α-, γ-, and δ-tocopherols, respectively, were obtained. The slopes and intercepts for the lines were:

α-tocopherol $Y = 40\,700 \times X + 25\,700$
γ-tocopherol $Y = 64\,200 \times X + 67\,900$
δ-tocopherol $Y = 64\,900 \times X + 460\,600$

Analysis of fresh unprocessed chicken breast muscle, with skin and all visible fat removed, indicated that the free α-tocopherol level ranged from 246 to 411 ng/g with a mean of 326 ng/g. Free γ-tocopherol levels ranged from 106 to 216 ng/g with a mean of 145 ng/g. The total mean unesterified tocopherol content in breast was 471 ng/g. Coefficient of variation between duplicate chicken samples was 6·8%. Literature values (McLaughlin & Weihrauch, 1979) would seem to indicate these tocopherol levels to be low for chicken. A value of 3400 ng/g for total tocopherol in raw chicken is often cited without indication of the parts of chicken which were analyzed. Skin, leg, thigh, and some organs contain levels of tocopherol that are four or more times greater than breast. Concentrations of tocopherol in other fresh poultry breast tissue appear to be considerably lower (turkey 900 ng/g, pigeon 600 ng/g (McLaughlin & Weihrauch, 1979)), and are comparable with those measured in this study. Seasonal variations, due to changes in the diet, also greatly influence tocopherol content (Piironen *et al.*, 1985). Saponification, sample preparation, and specificity of the detection system

(fluorimetry versus colorimetry (Emmerie–Engel reaction) all have a bearing on the levels of vitamin E that have been reported.

Recovery studies, where breast muscle was spiked with 480 ng of α-tocopherol, comparable with the level normally detected in this study, resulted in an average recovery of 92%.

The experimental irradiation conditions used in this study approximated those approved by the FDA. Breasts therefore were irradiated under aerobic conditions in heat-sealed (non-evacuated) air-permeable meat and poultry bags. To obtain additional insight regarding the effects of ionizing radiation, the samples were irradiated up to a dose level of 10 kGy, thus exceeding the 3 kGy limit set by the U.S. government. The effect of gamma radiation on the percentage reduction of α- and γ-tocopherol as a function of the dose is presented in Figs 3 and 4. Examination of the graph readily reveals the existence of a great deal of variability within the results. The data were subjected to an analysis of variance and regression analysis. Results from the statistical analysis found evidence of a significant linear decrease for both α- and γ-tocopherol with increasing dosage levels.

α-tocopherol: regression equation $r = -0{\cdot}5949$
$Y = 76{\cdot}51 - 2{\cdot}03 \times \text{Dose}$ $P < 0{\cdot}01$
γ-tocopherol: regression equation $r = -0{\cdot}4244$
$Y = 95{\cdot}00 - 2{\cdot}23 \times \text{Dose}$ $P < 0{\cdot}03$

Extrapolation of the data presented in Figs 3 and 4 indicates that irradiation of chicken at a dose level of 3 kGy (maximum level currently

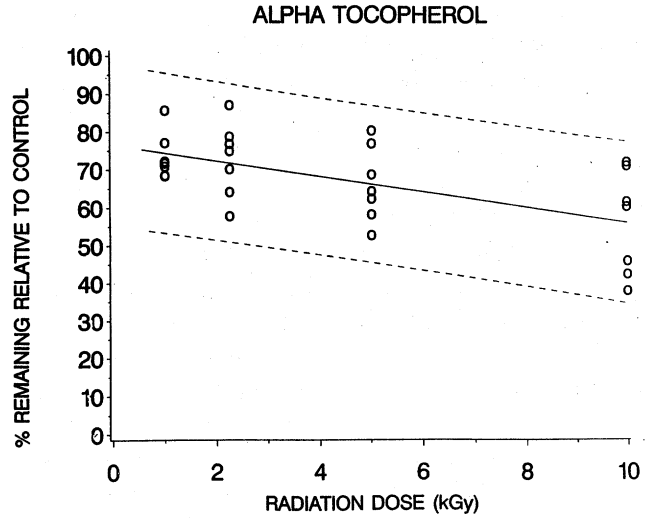

Fig. 3. Relationship between dose and concentration of free α-tocopherol in chicken breast relative to unirradiated control. Mean and 95% confidence limits. No. = 28.

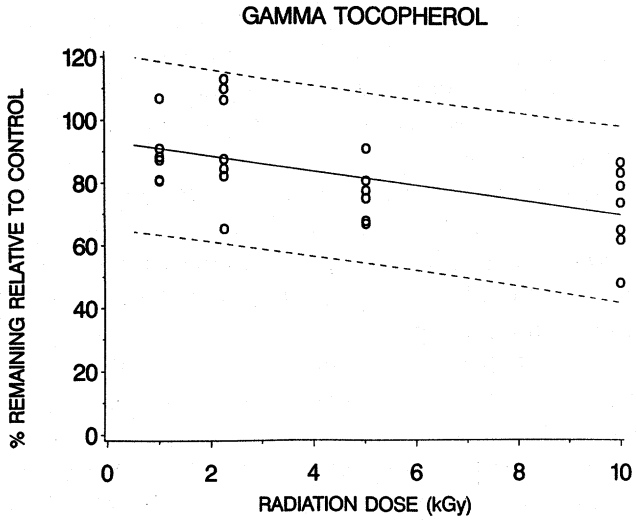

Fig. 4. Relationship between dose and concentration of free γ-tocopherol in chicken breast relative to unirradiated control. Mean and 95% confidence limits. No. = 28.

allowed) would result in an approximate 30% reduction in α-tocopherol and a 15% reduction in γ-tocopherol. A direct comparison with the findings of other investigators is not possible, since there have been very few studies conducted on the effects of radiation on tocopherol levels in meat. The most comparable work was by de Groot *et al.* (1972). In that study, which was submitted as a report to the Central Institute for Nutrition and Food Research, the authors measured the effect of 3 kGy and 6 kGy of radiation on several vitamins, including tocopherol, in chickens that had been irradiated, stored for 4–7 days at 5°C and cooked. In the first set of studies there was no apparent affect at 6 kGy but a 40% reduction at the lower 3 kGy dose. The second study indicated a 55% reduction at both levels. The authors noted wide variability between different batches of chickens, and between different treatments within the same batch. Their investigation concluded that overall (11 vitamins were studied) there was no distinct effect of ionizing irradiation on the vitamin content. In an article (Roussel, 1988) on irradiation of mechanically deboned dehydrated products (poultry, beef, pork, veal and fish), a brief statement mentioned that the level of vitamin E was 'diminished'. This report may be based on a French study (Anon., 1985) which stated that irradiation of mechanically separated meat at 5 kGy resulted in a 33–60% reduction in α- and γ-tocopherol. In a study on the effects of irradiation at different temperatures and in different gaseous atmospheres, Diehl (1979*b*) demonstrated an approximate 75% loss in α-tocopherol when pork was irradiated with 50 kGy at 0°C in air. That dose level was 17 times greater than currently allowed.

CONCLUSION

The data from this study indicate that irradiation of chicken breast in air, at 4°C, would result in a moderate decrease in α- and γ-tocopherol. At a dose of 3 kGy, a 15% loss of γ-tocopherol and a 30% loss of α-tocopherol could be expected. Since the tocopherols are the most labile of the lipophilic vitamins, no substantial reductions would be anticipated for the other more stable vitamins—A, D, or K (Knapp & Tappel, 1961*a*).

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Reference to brand or firm name does not constitute endorsement by the USDA over others of a similar nature not mentioned.

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